Reference: *Biol. Bull.*, 141: 350–363. (October, 1971)

ROLE OF SYMBIOTIC ALGAE (ZOOXANTHELLAE) IN CORAL CALCIFICATION

VICKI BUCHSBAUM PEARSE[1] AND LEONARD MUSCATINE

Department of Zoology, University of California, Los Angeles, California 90024

Members of many invertebrate groups live symbiotically with unicellular algae, but the symbiosis between corals and dinoflagellate algae (zooxanthellae) is especially interesting because it occurs in all species of tropical reef-building corals (see reviews by Droop, 1963; Yonge, 1963; McLaughlin and Zahl, 1966). Moreover, a significant effect of the algae on the physiology of corals has been clearly demonstrated and quantified: Corals with symbiotic algae calcify many times faster in light than in darkness, while corals which have lost their zooxanthellae calcify at rates which are slower and unaffected by light (Kawaguti and Sakumoto, 1948; Goreau, 1959; Goreau and Goreau, 1959). In the light, photosynthesis by zooxanthellae must somehow lead to higher rates of calcification by corals.

Three mechanisms have been proposed to explain how zooxanthellae influence coral calcification: (1) removal of carbon dioxide in photosynthesis directly favors chemical equilibria leading to the precipitation of calcium carbonate (Goreau, 1959); (2) algal removal of phosphates, which may act as crystal poisons, enhances crystallization of calcium carbonate (Simkiss, 1964a, 1964b); and (3) organic products of photosynthesis, either specific materials required for skeletogenesis, or nutrients or general energy sources supplied to the coral, permit faster calcification (Goreau, 1959; Wainwright, 1963). So far, there has been no experimental evidence which conclusively supports or eliminates one hypothesis or another.

One observation appears to be inconsistent with current ideas about the intimate relationship between algal photosynthesis and coral calcification. In the staghorn coral, *Acropora cervicornis* (Fig. 1), as in other branching forms, calcification rates are highest in the tips, decreasing progressively towards the base (Goreau and Goreau, 1959). However, very few symbiotic algae are found in the tips, their numbers increasing towards the base. Where abundant, they give the coral a deep brown color, contrasting sharply with the whiteness of the almost algae-free tips (Figs. 1 and 2). We undertook a study of the rapidly calcifying tips in order to clarify the problem of how the algae stimulate coral calcification rates.

Materials and Methods

Collection and incubation

Corals were collected from shallow reefs (1–3 meters) in Discovery Bay on the north coast of Jamaica and kept in running seawater for up to a few hours until the start of each experiment. All light experiments were run outdoors in natural light (500–2000 footcandles), but not in direct sunlight. Dark controls were run simultaneously in blackened boxes. In a few experiments, the tips of some of the coral branches were covered with opaque cylindrical caps, 10 mm long, 7 mm in diameter, covered with aluminum foil and lined with black plastic tape

[1] Present address: Hopkins Marine Station, Stanford University, Pacific Grove, California 93950.

(Fig. 3). Temperature in both light and dark was maintained at 26° ± 1° C. Coral branches cut off 30 mm from the tip were incubated in seawater to which radioisotope had been added, in shallow, transparent glass or plastic vessels. The vessels contained about 80 mg coral/ml seawater and were stirred occasionally during incubation. At the end of incubation, the corals were washed in fresh seawater to remove unused radiosotope, and sections of standard sizes were cut for processing. Several sections were sometimes pooled and counted together. Incubations lasted 2–4½ hours.

Labeling with calcium-45

To measure calcification, coral branches were incubated in seawater with 1–2 μc/ml $Ca^{45}Cl_2$. Sections were dissolved in hydrochloric acid, and aliquots of acid were plated on planchets, dried, and immediately assayed for radioactivity with a Nuclear Supplies Model SA 250 scaler and GM Lionel Anton 1007T thin end-window tube.

Labeling with carbon-14

To measure accumulation of organic carbon, coral branches were incubated in seawater with 1–3 μc/ml $NaHC^{14}O_3$. The sections were processed for organic carbon-14 determinations by two different methods. In some experiments, the samples were placed in hot 3 *N* KOH to remove the tissue, which was then homogenized in a glass tissue grinder and brought to known volume; aliquots were plated, dried, and assayed for radioactivity as above. In others, the corals were extracted several times with warm 80% ethanol, then with a mixture of absolute methanol and chloroform (2:1); these were combined as a "soluble" tissue fraction and acidified before counting. HCl was added to the insoluble residue to decalcify the skeleton, and the resulting tissue suspension was homogenized. Aliquots of both soluble and insoluble fractions were plated, dried, and counted as above.

Both calcification and accumulation of organic carbon are expressed as counts per minute (Ca^{45} or C^{14}) per microgram protein nitrogen in the sample, corrected for background and self-absorption. It should be noted that the values do not necessarily represent constant rates, but rather the total amount of calcium-45 or organic carbon-14 accumulated in a given period. Thus, absolute values can only be compared among controls within a given experiment, for which all experimental conditions were alike. Incubation time, light intensity and temperature probably constitute the most important variables among conditions in the different experiments.

Determination of protein nitrogen

Protein nitrogen values were determined by the method of Lowry, Rosebrough, Farr and Randall (1951). Commercially standardized bovine serum albumin was used as a standard. Pieces of coral were heated in concentrated ammonium hydroxide for about one hour. The tissue was then brought into suspension by gentle agitation, and the clean skeleton was removed. The tissue suspension was homogenized in a glass tissue grinder and brought to known volume; aliquots of this homogenate were taken for protein nitrogen determinations. Since it was technically difficult to determine both radioactivity and protein nitrogen from the same

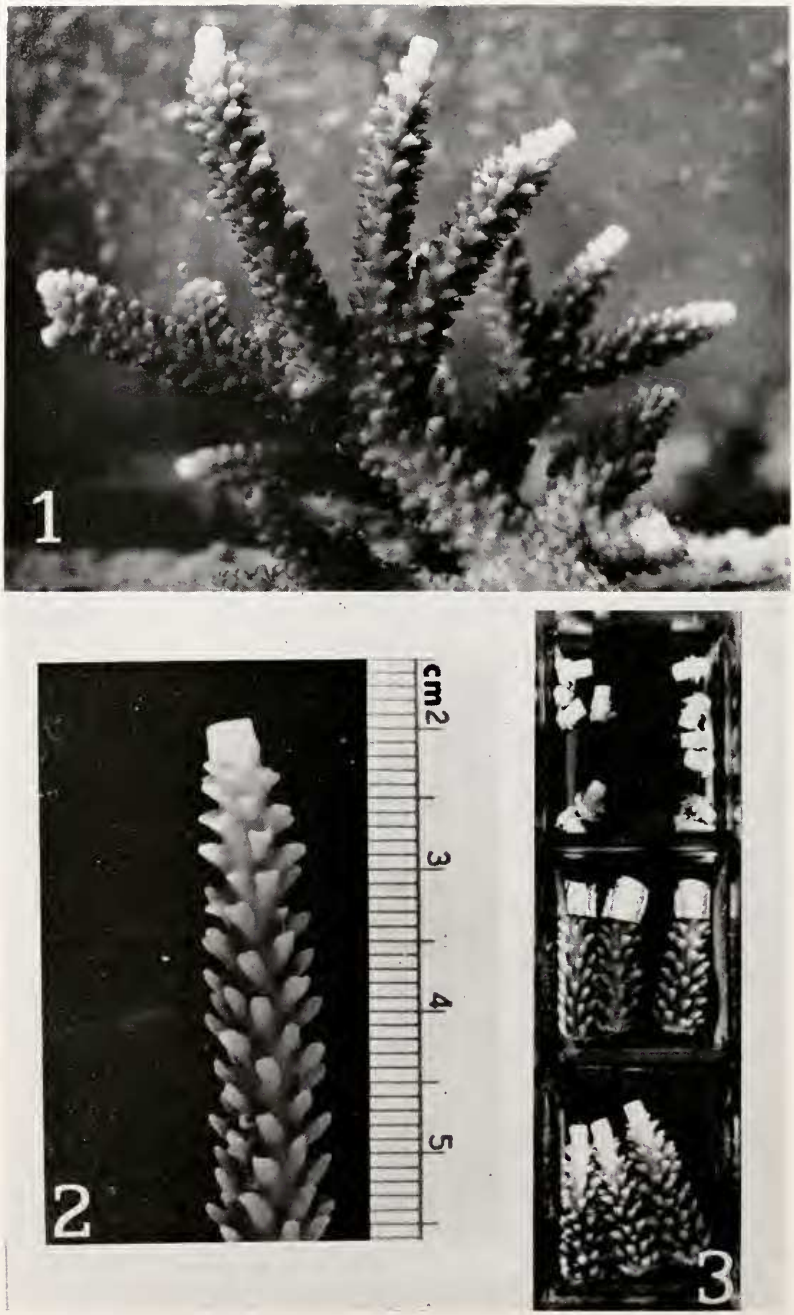

FIGURE 1. Small colony of the reef-building coral *Acropora cervicornis*. Each branch bears many lateral polyps and a single large terminal polyp. As the branch grows outward, new lateral polyps continually develop in a zone just below the terminal polyp and others are added at lower levels as the diameter of the branch increases. The deep brown color of the

experimental sample, protein nitrogen values were determined for a number of non-radioactive samples of standard sizes, and average protein nitrogen values were used in calculations.

Determination of chlorophyll

The relative amounts of zooxanthella chlorophyll in coral tissue were determined by spectrophotometric readings of pigment extractions, after the method of Richards with Thompson (1952; see also Strickland and Parsons, 1965). Standard sections of coral from three different regions of the branches were cut and placed in test tubes, 5 sections of each region per tube. To each tube, 3.0 ml 90% acetone and a drop of an aqueous suspension of $MgCO_3$ were added. The tubes were filled with nitrogen, tightly stoppered, and stored in a dark refrigerator for 24 hours, with occasional stirring. The final extracts were centrifuged, and optical density (uncorrected values, determined directly from the extracts) was read in a Bausch and Lomb Spectronic 20 spectrophotometer at 630 nm for chlorophyll *c* and 665 nm for chlorophyll *a* against a blank containing 90% acetone.

Chromatographic procedures

Two-dimensional radiochromatography and identification of labeled unknowns were carried out as described previously (Muscatine, 1965; Muscatine and Cernichiari, 1969), following the procedure of Benson, Bassham, Calvin, Goodale, Haas and Stepka (1950). The procedure for deacylation of lipids is also described by Muscatine and Cernichiari (1969).

Results

Gradients in coral branches

In the first series of experiments, chlorophyll, protein nitrogen, dry weight (mostly calcium carbonate), and calcification in light and dark (as calcium-45 labeling) were measured in three successive sections of coral branches from the tip downwards (Fig. 4). The term "tip" designates the terminal polyp only. In calcification experiments, intact 30 mm coral branches were incubated in seawater with added calcium-45 in light and dark, and sections of the branches were cut at the end of the incubation period.

Figure 4 shows two features which are immediately apparent by simply examining a coral branch (see also Goreau, 1963). First, the amount of algal pigment in the branch increases from the tip towards the base (Fig. 4A). Although the zooxanthellae were never counted, tissue smears revealed a gradient in the numbers of algal cells which paralleled the data for chlorophyll. Second, the dry weight per protein nitrogen of each section (tissue plus skeleton) increases from the tip towards the base (Fig. 4B), as the tissues constitute a greater proportion

---

tissue is due to large numbers of symbiotic zooxanthellae; the white tips contain very few zooxanthellae.

Figure 2. A single branch, enlarged to show the details of algal distribution and developing polyps near the tip.

Figure 3. Incubation chambers containing (*top*) 5-mm pieces bearing "isolated" tips, (*center*) branches with opaque caps covering the tips to shield them from light, (*bottom*) 30-mm branches with intact tips.

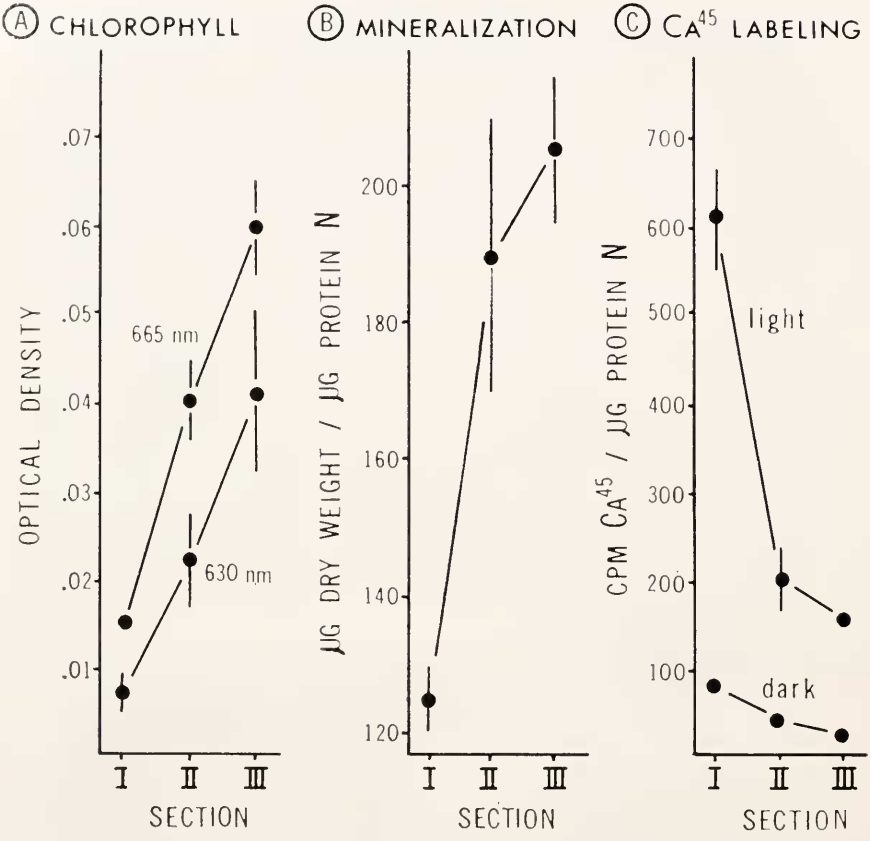

FIGURE 4. Gradients in coral branches. (Mean values ± one standard deviation). *Section I:* 0–3 mm, terminal polyp only, lightly calcified, few zooxanthellae. *Section II:* 3–6 mm, developing lateral polyps, well calcified, more zooxanthellae. *Section III:* 6–9 mm, fully-developed lateral polyps, well calcified, abundant zooxanthellae.

of the total weight in the soft, lightly calcified tip than in the heavily calcified basal portions. This suggests that calcification should take place most rapidly in the tip, the rate decreasing basally, as has indeed been found by Goreau and Goreau (1959) and as our calcium-45 data confirm (Fig. 4C). The curves in Figures 4B and 4C are thus approximately reciprocal.

The intrinsic gradient in calcification rates, decreasing from the tip downwards, is expressed by the dark values in Figure 4C. We expected that in the light, this gradient would be tempered or even reversed, since the enhancement of calcification rates by light would be greatest in the basal portion of the branch, where zooxanthellae are most abundant, while the white tips would be least affected. Surprisingly, however, we found the reverse: The intrinsic calcification gradient was actually reinforced in the light, and the effect of light on calcification, reflected in the light/dark ratios calculated for each section (Fig. 6, solid curve), was consistently greatest in the tip (Section I), where zooxanthellae were rare. The effect

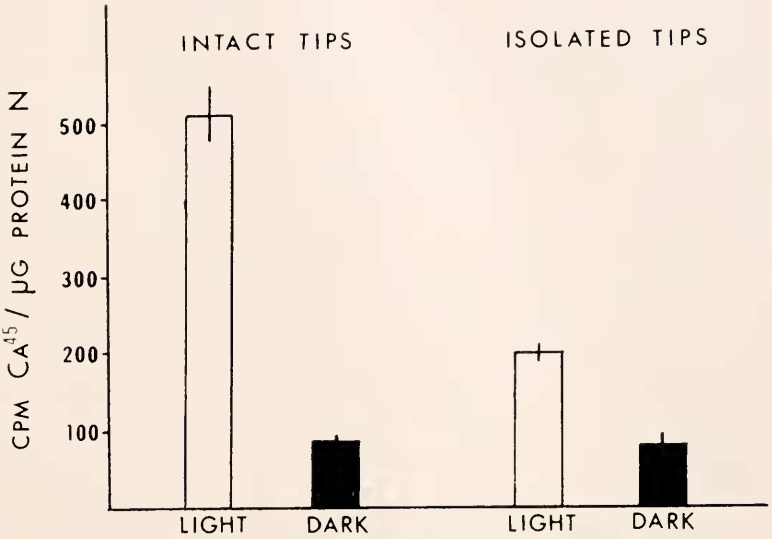

FIGURE 5. Calcification of intact and isolated tips, in light and dark. (Mean values ± one standard deviation, each representing 9 coral tips pooled in 3 lots of 3 tips each.)

of light was consistently least in Section II, with intermediate numbers of zooxanthellae. Light/dark values varied in different experiments, but this pattern was constant.

Measurements of calcification

To explain the high light/dark ratios for calcification in the algae-poor tip, we hypothesized that algae in lower portions of the branch might be enhancing calcification rates in the tip. To test this possibility, we ran a second series of calcification experiments in which some 30 mm branches were incubated with the tips intact as before, while in other branches, the terminal 5 mm (*i.e.*, the tip plus a portion of Section II) was cut off and incubated alone. A brown zone of zooxanthellae was usually visible in the bases of these 5 mm pieces (see Figs. 2 and 3), so the tips were not completely isolated from adjacent algae but were cut off from the bulk of the algae in the branch. All were incubated with calcium-45 in light and dark. After incubation, only the 3 mm tip (terminal polyp, Section I) was cut from each piece for calcium-45 determination. The results of one of these experiments are presented in Figure 5.

In the dark, intact and isolated tips appeared to calcify equally. The dark controls thus serve also as controls for possible damage or other effects of isolating the tips. In the light, calcification in intact tips increased six fold, while calcification in isolated tips increased only two fold over dark levels.

To determine the effect of darkening the tips only, another calcification experiment was run in which the tips of some of the coral branches were covered with opaque caps (see Methods and Fig. 3). In this experiment, summarized in the first half of Table I, intact and isolated tips show the same relationships to dark controls as before. Calcification in intact, capped tips on illuminated branches was

greater than in tips of branches which were wholly dark, consistent with the hypothesis of translocation. Dark controls with and without caps indicated that the caps themselves had no apparent effect on calcification. Isolated tips placed inside caps in the light calcified at the same levels as dark controls, confirming that the caps did effectively exclude light.

To resolve the paradox of finding the highest light/dark ratios in the algae-poor tips, Sections II and III from branches with intact tips were also analyzed in this experiment. The light/dark ratios of Sections I, II and III (Fig. 6, solid line) followed the usual pattern, with the tip showing the highest ratio and Section II, the lowest. However, if the light and "dark" (capped) values for isolated tips (Table I) are substituted (Fig. 6, dotted line), the tip then has the lowest light/dark ratio. The ratios increase towards the base, paralleling the increase in abundance of algae (Fig. 4A), as would be expected. The seemingly great effect of light on tip calcification in the almost complete absence of algae must therefore depend on algae present in lower portions of the branch. When the tip is experimentally isolated from the bulk of the algae, the effect of light on calcification is much diminished.

The results of our calcification experiments seemed to indicate, in summary, that: (1) There is a gradient in calcification rates from the tip downwards, in both light and dark (Fig. 4C); (2) intact tips show the greatest light enhancement of calcification (Fig. 6, solid curve), although they contain fewer zooxanthellae than lower portions (Fig. 4A), but (3) if values for isolated tips are used, the light enhancement gradient parallels the algal gradient, as would be expected (Fig. 6, dotted curve). (4) Intact tips calcify faster than isolated tips in the light, but not in the dark (Fig. 5); and (5) light enhancement of calcification is still seen when the tip itself is dark and only lower portions of the branch are illuminated (Table I).

These results all support the hypothesis that rates of calcification in the coral tips were stimulated in some way during photosynthesis by zooxanthellae in lower portions of the branch. Since transfer of materials from zooxanthellae to the tissue

TABLE I

Calcification and organic carbon in intact and isolated coral tips; effect of darkening tips only. (Each Ca^{45} value represents 3 tips pooled together. Each C^{14} value represents 6 tips pooled together; C^{14}-labeled tissue was fractionated by extraction with alcohol-chloroform mixtures)

	cpm Ca^{45}/μg protein N	cpm C^{14}/μg protein N		
		Soluble	Insoluble	Total
Intact tips, in light	160, 160	23.8	12.1	35.9
Isolated tips, in light	79, 80	16.9	6.7	23.6
Intact tips, in light, with caps	85, 93	5.2	2.9	8.1
Intact tips, in dark	51, 54	2.0	1.7	3.7
Intact tips, in dark, with caps	52, 57			
Isolated tips in light, with caps	52, 57			

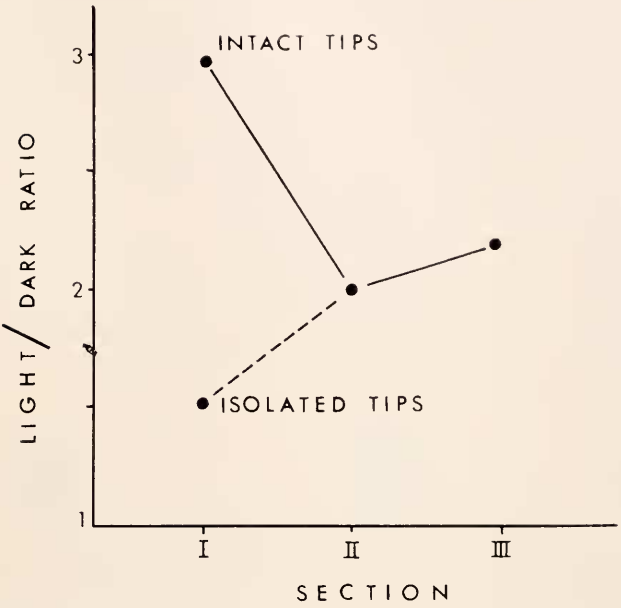

FIGURE 6. Light/dark ratios for calcification: intact vs. isolated tips. (Sections as in Fig. 4.)

of a coral had been demonstrated previously (Muscatine and Cernichiari, 1969), we hypothesized further that calcification might be stimulated through an organic photosynthetic product, translocated to the tip.

Measurements of accumulation of organic carbon

To test the possibility that photosynthetically fixed organic carbon was translocated from the algae to the coral tip, 30 mm branches with intact tips and isolated tips on 5 mm pieces (Fig. 3) were incubated in seawater containing added carbon-14 as sodium bicarbonate, in light and dark. After incubation the 3 mm tips only were removed for assay, just as in the calcium experiments above. The results of one of the carbon-14 experiments in which the tissue was removed in KOH (see Methods) are presented in Figure 7. Dark values probably represent primarily heterotrophic fixation by coral tissue, and were not significantly different in intact and isolated tips. In the light, however, intact tips contained significantly more organic carbon-14 than isolated ones.

In another experiment, summarized in the second half of Table I, the tissue components were fractionated by extraction with alcohol-chloroform mixtures (see Methods). As it was necessary to pool the samples, only single values were obtained and the level of significance of the differences observed is uncertain. However, the results were uniformly consistent with those from whole tissue. Intact tips contained more organic carbon-14 than isolated ones, and capped tips with illuminated bases contained more organic carbon-14 than dark controls. These differences appeared in both soluble and insoluble fractions.

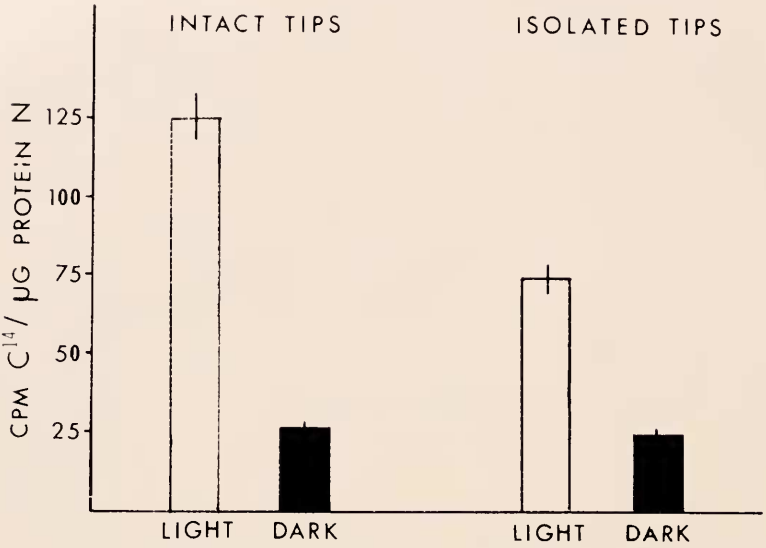

FIGURE 7. Organic carbon-14 in intact and isolated tips, in light and dark; whole tissue in KOH. (Mean values ± one standard deviation, each representing 9 coral tips pooled in 3 lots of 3 tips each.)

It is likely that our values from 5-mm "isolated" tips consistently overestimate both calcification and accumulation of organic carbon in the tip proper, because (1) the few algae in the tips themselves are included in our C^{14}-accumulation data, although their contained products are not strictly within the coral tissue, and (2) the bases of the "isolated" tips include the first developing lateral polyps with substantial numbers of zooxanthellae, the tip proper being too soft and fragile to incubate alone, and although these algae are not included in the final assay, their products may be translocated to the tip during incubation (compare light and dark isolated controls, Fig. 7). Values for capped tips, on the other hand, probably underestimate calcification and organic carbon accumulation (Table I) in a darkened tip, because the 10-mm caps, necessary to shade the tip completely, also partially shade adjacent lateral polyps with their contained zooxanthellae. Thus the differences between intact and isolated tips are consistently minimized by the experimental conditions, and the differences between uncapped and capped tips are exaggerated.

In addition, although the caps appeared to have no direct effect on calcification (compare capped and uncapped dark controls, Table I), capped branches released more than three times as much fixed organic carbon-14 into the seawater medium as did the uncapped branches. In two experiments in which the seawater medium was assayed, the total fixed carbon-14 in tips + medium was approximately the same in both capped and uncapped controls, but uncapped branches released only 18% and 21% of the total into the medium while capped branches released 54% and 53%. Neither the mechanism nor the significance of this release to the medium is understood. Perhaps increased release resulted from cell damage by the caps, but this seems unlikely, as the caps fitted very loosely (see Fig. 3) and, as noted

above, appeared to have no direct effect on calcification (Table I). It is possible also that darkening of the tip inhibits axial translocation and leads instead to release of translocated material to the medium; or that translocated products accumulate in the confined medium inside the cap and reach concentrations which inhibit further translocation, again leading to increased release. The explanation of this phenomenon awaits further investigation, but it is probably responsible for the fact that capped tips accumulate less organic carbon than one might expect even from the minimal differences measured between intact and isolated tips. Thus although capped tips compare unfavorably with free, intact ones in the light, capped tips with illuminated bases do exceed tips of dark controls in both calcification and accumulation of organic carbon (Table I), consistent with the hypothesis that axial translocation of organic photosynthetic products somehow stimulates calcification rates in the tip.

Analysis of C^{14}-labeled products

In an effort to localize the label into as few products as possible, a "pulse-chase" experiment was also run. Coral branches were incubated in the light for 30 minutes in seawater containing 3 μc/ml $NaHC^{14}O_3$. All tips were intact, and all were covered with opaque caps in order to minimize photosynthesis by the few algae in the tips. At the end of the "pulse," the branches were quickly washed in fresh seawater, and a zero-time sample of tips was processed immediately to yield soluble and insoluble fractions (see Methods). Tips were isolated (cut off at 5 mm) from half of the remaining branches; the rest were left intact. Both groups were placed in the dark in fresh seawater without radioisotope for 3 hours and then processed as usual into soluble and insoluble fractions. The values obtained (cpm organic C^{14}/μg protein N, each value representing 6 tips pooled together) from tips at zero-time and from intact and isolated tips at 3 hours were, respectively, as follows: Soluble: 4.8, 5.9, 3.6; Insoluble: 2.7, 2.9, 2.6; and Total: 7.5, 8.8, 6.2.

In the intact tips, carbon-14 activity appeared to increase with respect to the zero-time sample, suggesting that some labeled product or products, photosynthetically fixed by the algae in lower portions of the branches during the light incubation, continued to move distally into the intact tips during the dark period. The isolated tips showed a loss of labeled carbon, compared to the zero-time sample, probably due primarily to respiration over the 3-hour dark period. The difference between intact and isolated tips appeared most evident in the alcohol-chloroform soluble fraction. Soluble material from intact, isolated, and intact capped tips, incubated with $NaHC^{14}O_3$ in the light, was therefore analyzed by two-dimensional paper chromatography in order to identify the labeled product or products. The results are given in Table II.

By far the most carbon-14 was contained in lipids, followed by glycerol and glucose. This large percentage of label in lipids suggested the likelihood of glycerol translocation since this compound is known to be released *in vivo* (Muscatine and Cernichiari, 1969), followed by synthesis of lipids in the coral tissue. Deacylation showed that lipids were indeed labeled only in the glycerol moiety.

Intact, isolated, and intact capped tips were incubated in anticipation that their labeled contents would include different proportions of the translocated materials.

TABLE II

Per cent distribution of organic C^{14}-labeled products in the soluble fraction of coral tissue. (Each value represents 12 coral tips pooled together)

Compound	Intact tips	Isolated tips	Intact tips, capped
glucose	6.78	6.48	5.15
glutamine	2.21	1.13	2.94
alanine	0.81	0.45	0.76
glycerol	8.64	7.88	6.04
lipids	80.18	83.32	81.17
unknown	0.53	1.31	3.60

However, there appeared to be no qualitative differences in carbon-14 distribution among them, suggesting that translocation from algae adjacent to the tips, even in isolated tips as discussed above, may be a much greater source of labeled carbon than algae within the tips themselves.

DISCUSSION

The results of our calcium-45 experiments suggest that calcification in the tip of a branch of *Acropora cervicornis* is increased in the light as a result of photosynthesis by symbiotic algae farther down in the branch. If so, the mechanism by which the zooxanthellae stimulate calcification must be one which can act over some distance. We put forward for testing the hypothesis that some organic product or products of algal photosynthesis, translocated axially to the coral tissue, in original or altered form, stimulate calcification, especially in the tip.

Our carbon-14 experiments indicate that axial translocation of algal products does take place in *Acropora*. It has been shown (Muscatine and Cernichiari, 1969) that, in the coral *Pocillopora damicornis*, 35–50% of the total photosynthetic product is excreted by the zooxanthellae, primarily as glycerol. In the coral tissue, the glycerol is converted largely to lipids, and the skeletal organic matrix has a substantial lipid component, consisting mostly of cetyl palmitate (Young, 1969; S. D. Young, J. D. O'Connor and L. Muscatine, in preparation). In *Acropora*, Lewis and Smith (1971) found that glycerol, glucose and alanine appear to be translocated *in vivo* by the zooxanthellae, and we also found glycerol (free and in lipid) and glucose to be the major labeled products in the coral tips (Table II). The chemistry of the skeletal organic matrix of *Acropora* has not been studied, and we did not attempt to recover it for separate analysis. However, if it is similar to those in other corals, the algal products could provide the raw materials for several major components: the glycerol could be incorporated into lipids; the glucose, into N-acetylglucosamine (see also Wainwright, 1963); and the alanine, into protein. All of these could also be used less specifically as general sources of energy for skeletogenesis.

We suggest that translocated algal products may enhance calcification rates in corals by serving either as specific substrates in the organic matrix or as general energy sources. The fact that calcification and translocation are diminished approximately to the same extent when the *Acropora* tip is isolated from the bulk of the zooxanthellae in the branch (Figs. 5 and 7) provides support for this hypothe-

sis. But there is still no direct evidence for the ability of algal products to stimulate calcification in any coral.

Goreau (1959) found that even in the dark, normal corals with zooxanthellae sometimes calcified 2 to 3 times faster than corals which had lost their zooxanthellae. He suggested that translocation of organic products from zooxanthellae may have been responsible for this dark enhancement of calcification. Since our pulse-labeling experiment indicated that some translocation of organic products to *Acropora* tips did continue in the dark, one might expect small differences between intact and isolated tips in the dark as well as in the light. Simkiss (1964a, 1964b) also suggested that zooxanthellae might stimulate calcification in the dark by absorbing phosphates which are potential inhibitors of calcification. Yamazato (1966) found that phosphate uptake by the coral *Fungia scutaria* did continue in the dark, although dark uptake rates were only 14% of those in the light. We found no significant differences in calcification between intact and isolated tips in the dark; if either translocation or phosphate uptake is in fact related to calcification, the quantities involved were insufficient to show measurable differences in the dark under our experimental conditions.

Translocation may also account for another seeming paradox in *Acropora* data. Goreau (1963) calculated approximately equal productivity values for terminal and lateral polyps of *Acropora* on the basis of organic carbon-14 measurements. The relatively high values for terminal polyps were unexpected in view of their much lower chlorophyll content and fewer zooxanthellae, and Goreau proposed an explanation in terms of shading effects. We suggest the alternative possibility that his values for terminal polyps may have included a large component of translocated organic carbon.

Goreau (1963) also compared calcification in corals and coralline algae, particularly in relation to productivity. Recent studies have demonstrated (Pearse, in preparation) several striking parallels between the characteristics of coral calcification discussed here and calcification in coralline algae of the genus *Bossiella* and probably *Amphiroa:* (1) the terminal portion of each branch of a plant is less heavily calcified than more basal portions; (2) there is a decreasing gradient in calcification rates from the terminal to more basal portions, in both light and dark; (3) calcification rates are greater in light than in darkness (see also Goreau, 1963); (4) the increase in calcification rates in the light is greatest in the terminal portion; and (5) the terminal portion appears less heavily pigmented, often nearly white, compared with more basal portions. Although there is no information about translocation in these plants yet, our studies on corals suggest that it is a likely possibility.

The hypothesis that zooxanthellae enhance coral calcification rates through translocation of organic carbon produced in photosynthesis in no way excludes the carbon dioxide hypothesis of Goreau or the phosphate hypothesis of Simkiss. Any or all of these mechanisms may operate in many corals. However, since the mechanisms proposed by Goreau and Simkiss both depend on the maintenance of a strong concentration gradient of carbon dioxide and phosphate respectively, they seem less likely to be effective where the site of calcification is at some distance from the bulk of the zooxanthellae, as in the rapidly calcifying tips of branches of *Acropora.*

We dedicate this paper to the late T. F. Goreau [2] in gratitude for the stimulating leads he provided in his extensive work with corals and for making available to us the facilities of the UWI-SUNY Marine Laboratory in Discovery Bay, Jamaica. We thank Mrs. E. Cernichiari and Mr. N. Copland for technical and administrative assistance; Mr. R. Pool for help in collecting corals; Mr. H. Reiswig and Mrs. S. Sidman for assistance with figures; Drs. D. C. Smith and D. H. Lewis for many helpful suggestions during the course of this work; and Dr. J. S. Pearse for critical reading of the manuscript. Financial support was provided by a postdoctoral fellowship from the National Institutes of Health (to V. B. P.) and National Science Foundation grant GB-3728 (to L. M.).

Summary

1. In branches of the coral *Acropora cervicornis*, the abundance of symbiotic algae (zooxanthellae) increases from tip to base, while active calcification decreases. Light enhancement of calcification rates is, paradoxically, greatest in the algae-poor tips of branches.

2. Calcium-45 experiments on intact and isolated tips of the coral branches suggest that light enhancement of calcification in the algae-poor tip results from photosynthesis by zooxanthellae farther down in the branch.

3. Carbon-14 experiments indicate that organic products of algal photosynthesis are translocated to the coral tip. The main carbon-14 labeled products in the tip are lipids, glycerol and glucose.

4. Our data are consistent with the hypothesis that translocated algal products enhance coral calcification rates.

LITERATURE CITED

Benson, A. A., J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, 1950. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. *J. Amer. Chem. Soc.*, **72**: 1710–1718.

Droop, M. R., 1963. Algae and invertebrates in symbiosis. Pages 171–199 in P. S. Nutman and B. Mosse, Eds., *Symbiotic Associations, Symposium Society General Microbiology. Vol. 13.* Cambridge University Press, London.

Goreau, T. F., 1959. The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. *Biol. Bull.*, **116**: 59–75.

Goreau, T. F., 1963. Calcium carbonate deposition by coralline algae and corals in relation to their roles as reef-builders. *Ann. New York Acad. Sci.*, **109**: 127–167.

Goreau, T. F., and N. I. Goreau, 1959. The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. *Biol. Bull.*, **117**: 239–250.

Kawaguti, S., and D. Sakumoto, 1948. The effect of light on the calcium deposition of corals. *Bull. Oceanog. Inst. Taiwan*, **4**: 65–70.

Lewis, D. H., and D. C. Smith, 1971. The autotrophic nutrition of symbiotic marine coelenterates with special reference to hermatypic corals. I. Movement of photosynthetic products between the symbionts. *Proc. Roy. Soc. London, Series B*, **178**: 111–129.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265–275.

[2] See the paper by the late T. F. Goreau, N. I. Goreau and C. M. Yonge on pages 247 to 260 of this issue.—*Editor.*

McLaughlin, J. J. A., and P. A. Zahl, 1966. Endozoic algae. Pages 257–297 in S. M. Henry, Ed., *Symbiosis, Vol. 1.* Academic Press, New York.

Muscatine, L., 1965. Symbiosis of hydra and algae. III. Extracellular products of the algae. *Comp. Biochem. Physiol.*, **16**: 77–92.

Muscatine, L., and E. Cernichiari, 1969. Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biol. Bull.*, **137**: 506–523.

Richards, F. A., with T. G. Thompson, 1952. The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for the estimation of plankton pigments. *J. Mar. Res.*, **11**: 156–172.

Simkiss, K., 1964a. Phosphates as crystal poisons of calcification. *Biol. Rev.*, **39**: 487–505.

Simkiss, K., 1964b. Possible effects of zooxanthellae on coral growth. *Experientia*, **20**: 140.

Strickland, J. D. H., and T. R. Parsons, 1965. A manual of seawater analysis. *Fish. Res. Board, Can. Bull.*, **125**: 1–203.

Wainwright, S. A., 1963. Skeletal organization in the coral, *Pocillopora damicornis*. *Quart. J. Microscop. Sci.*, **104**: 169–183.

Yamazato, K., 1966. Calcification in a solitary coral, *Fungia scutaria* Lamarck in relation to environmental factors. *Ph.D. thesis, University of Hawaii*, Honolulu, Hawaii, 130 pp.

Yonge, C. M., 1963. The biology of coral reefs. Pages 209–260 in F. S. Russell, Ed., *Advances in Marine Biology, Vol. 1.* Academic Press, New York.

Young, S. D., 1969. Studies on the skeletal organic material in hermatypic corals with emphasis on *Pocillopora damicornis*. *Ph.D. thesis, University of California, Los Angeles.* 114 pp.